

Biospecimen Collection – Standard Operating Procedure

Consent

Please ensure patient/family have appropriately consented for the samples being collected and for ethically-approved research uses.

Samples

These may include tissue or circulating ('non-invasive') biospecimens. In addition to whole blood, the latter acellular samples may include serum, plasma, cerebrospinal fluid (CSF), urine, pleural fluid and ascites.

Sample processing

Fresh frozen tissue:

- Inform the Pathology team on operating room (OR) service upfront which specimens will require collection for the Biobank (since this will affect the way the specimen is handled on the day)
- Inform the surgical team to deliver the specimen with the accompanying requisition as fast as possible to the Pathology for collection (to reduce to the minimum the cold ischemia time)
- Collect a tissue fragment of the tumor (and/or adjacent organ parenchyma, if appropriate), of approximately 0.5cm, assuring that such collection does not preclude the proper diagnosis and staging of the patient. Use clean instruments in all instances. Avoid areas of macroscopically obvious necrosis (collect from areas with apparent vital tumor tissue)
- If feasible on that day, perform a frozen section of the fragment on a cryostat to confirm presence of viable tumor histologically (if not possible on that day, this can be done afterwards)
- Place the tissue fragment in the chosen sample container for snap freezing (cryovial, cryomold, routine histological cassette, other), appropriately labeled (local inventory code, barcode, other)
- Snap-freeze the tissue (pre-cooled isopentane, liquid nitrogen, commercially available freezing system, etc)
- Store the tissue in a -80C freezer
- See [Martins AT, Carneiro I, Monteiro-Reis S, Lobo J, Luís A, Jerónimo C, Henrique R. Frozen in Translation: Biobanks as a Tool for Cancer Research. Intellect Disabl Diagn J, 2015 Aug 3;\(2\):51-62. DOI: 10.6000/2292-2598.2015.03.02.2 for further details](#)

Formalin-fixed paraffin-embedded tissue:

- Whenever possible, collect a "twin fragment" of tissue for formalin fixation and paraffin embedding (i.e., bisect the tissue selected for snap freezing in two halves, selecting one half for formalin fixation and the twin half for freezing)
- Place the selected tissue fragment in a routine histological cassette, properly labeled (local inventory code, barcode, other), and place it in formalin for routine fixation (10% phosphate-buffered formalin). Annotate the time of entering formalin (and later the time of exiting formalin). The volume of formalin in the container should be 15 to 20 times the volume of the tissue
- Fixation time should be of minimum 6-8h and maximum 72h, at room temperature. After fixation, process the tissue for paraffin embedding as routinely done in local Pathology Department
- Store the blocks in air temperature room
- See [Martins AT, Carneiro I, Monteiro-Reis S, Lobo J, Luís A, Jerónimo C, Henrique R. Frozen in Translation: Biobanks as a Tool for Cancer Research. Intellect Disabl Diagn J, 2015 Aug 3;\(2\):51-62. DOI: 10.6000/2292-2598.2015.03.02.2 for further details](#)

Serum (for downstream microRNA analysis):

- Whole blood is collected in e.g., a standard gel separator tube (e.g., hospital electrolytes tube) or equivalent
- Sample is allowed to clot for 30 minutes (or as per the manufacturer's instructions)
- Sample is then centrifuged down at 2,500-3,000 x g for 10 minutes at room temperature (or as per the manufacturer's instructions) within four hours of collection
- This causes the blood cells to collect below the gel layer, and then the supernatant (serum; upper straw-colored clear fluid layer) is aliquoted off into fresh, appropriately labelled and dated 1ml tube(s), not containing any other components/reagents, and stored at -80C, until required for use.
- Avoid collecting obviously macroscopically bloodstained samples
- See [Murray et al, "Future-Proofing" Blood Processing for Measurement of Circulating miRNAs in Samples from Biobanks and Prospective Clinical Trials, Cancer Epidemiology, Biomarkers & Prevention, 2018](#) and [Murray et al, A Circulating MicroRNA Panel for Malignant Germ Cell Tumor Diagnosis and Monitoring, Methods in Molecular Biology, 2021](#) for further details.

Plasma (for downstream microRNA analysis):

- Whole blood is collected in e.g., an ethylenediaminetetraacetic acid (EDTA) tube or equivalent
- Samples is then processed within one hour of collection
- Sample is centrifuged at 1,600 x g for 10 minutes at room temperature (or as per the manufacturer's instructions)
- This causes the blood cells to form a layer at the bottom of the tube, and then the supernatant (plasma; upper straw-colored clear fluid layer) is aliquoted off into fresh, appropriately labelled and dated 1ml tube(s), not containing any other components/reagents, and stored at -80C, until required for use.
- Avoid collecting obviously macroscopically bloodstained samples
- See [Murray et al, "Future-Proofing" Blood Processing for Measurement of Circulating miRNAs in Samples from Biobanks and Prospective Clinical Trials, Cancer Epidemiology, Biomarkers & Prevention, 2018](#) and [Murray et al, A Circulating MicroRNA Panel for Malignant Germ Cell Tumor Diagnosis and Monitoring, Methods in Molecular Biology, 2021](#) for further details.

Plasma for downstream circulating tumour DNA (ctDNA) analysis]:

- The separated plasma, as aliquoted off above after the 1,600 x g centrifugation step, is subjected to a secondary centrifugation of 14,400 x g for 10 minutes at room temperature, as per standard protocol conditions for preparing plasma for optimal ctDNA analysis
- The supernatant is aliquoted off into fresh, appropriately labelled and dated 1ml tube(s), not containing any other components/reagents, and stored at -80C, until required for use.
- Avoid collecting obviously macroscopically bloodstained samples
- Such samples need to be clearly labelled to highlight that they have been subject to a second, high-speed centrifugation, as these samples are not optimal for downstream microRNA studies
- See [Murray et al, "Future-Proofing" Blood Processing for Measurement of Circulating miRNAs in Samples from Biobanks and Prospective Clinical Trials, Cancer Epidemiology, Biomarkers & Prevention, 2018](#) and [Murray et al, A Circulating MicroRNA Panel for Malignant Germ Cell Tumor Diagnosis and Monitoring, Methods in Molecular Biology, 2021](#) for further details.

CSF (for downstream microRNA or ctDNA studies) or other similar biospecimens (pleural fluid, ascites, urine etc):

- Any volume of CSF (or other biospecimen fluid) may be useful for molecular interrogation; note that as little as 50-200 μ l may be used for study, so small volume samples obtained from lumbar puncture can still be stored
- If CSF is taken at neurosurgical intervention for e.g., raised intracranial pressure, up to 2000 μ l or more may be stored.
- Such samples should be centrifuged at 1,000 x g for 10 minutes at room temperature within four hours of collection, so that any cells collect at the bottom of the tube, then supernatant (cell-free CSF or biofluid) is aliquoted off into fresh, appropriately labelled and dated 1ml tube(s), not containing any other components/reagents, and stored at -80C.
- Avoid collecting obviously macroscopically bloodstained samples